Supplementary Materials for

Quantification of antiviral drug tenofovir (TFV) by surface enhanced Raman spectroscopy (SERS) using cumulative distribution functions (CDFs)

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This file contains the following contents:
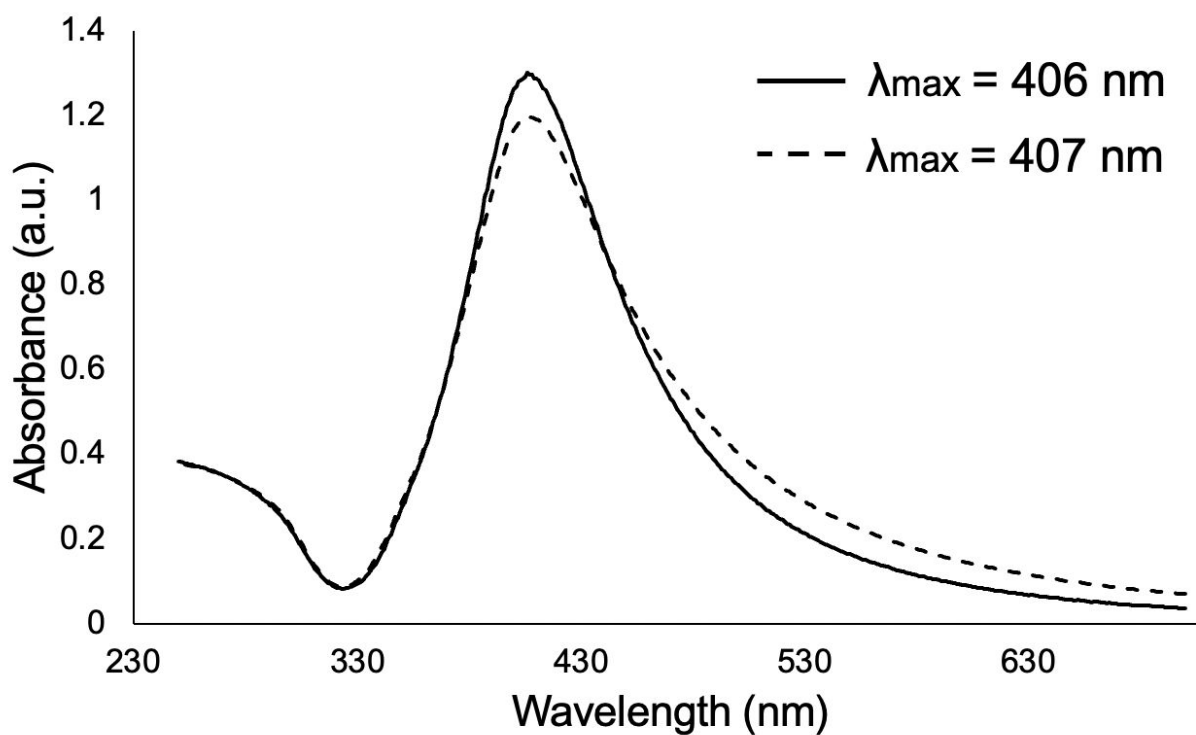Figure S1 – S5, Table S1

Fig. S1. Corrected UV- vis spectra of the Ag colloidal nanoparticle suspensions used in this study. These spectra were acquired using a Thermo Scientific Evolution 201 UV-Visible Spectrophotometer.
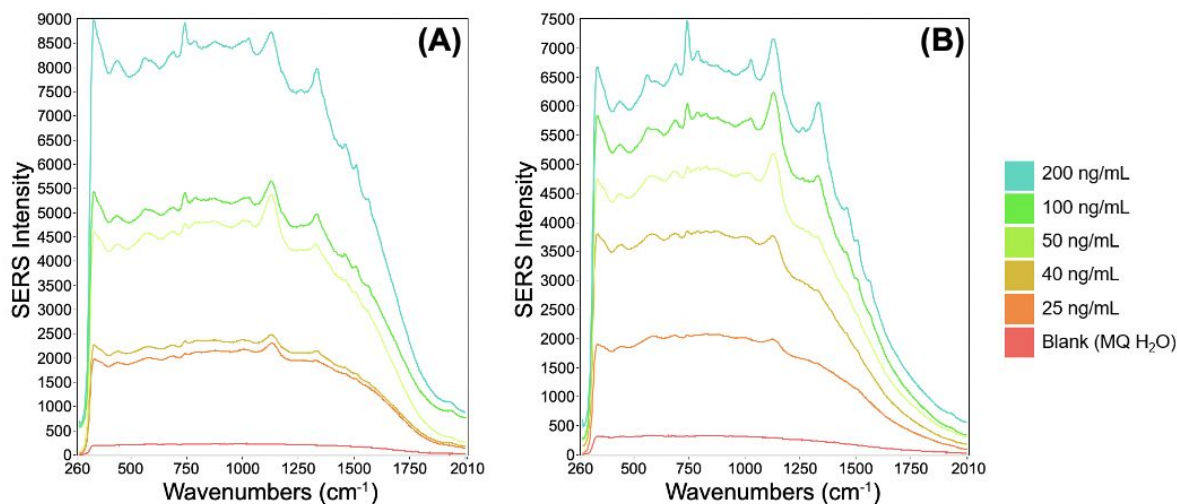
Fig. S2. SERS spectra from the **(A)** single deposition experiment and **(B)** double deposition experiment where each spectrum is an average of 100 spectra (20 spectra from each replicate). A 7- point Savitzsky- Golay smoothing function was applied. Spectra were offset for clarity.
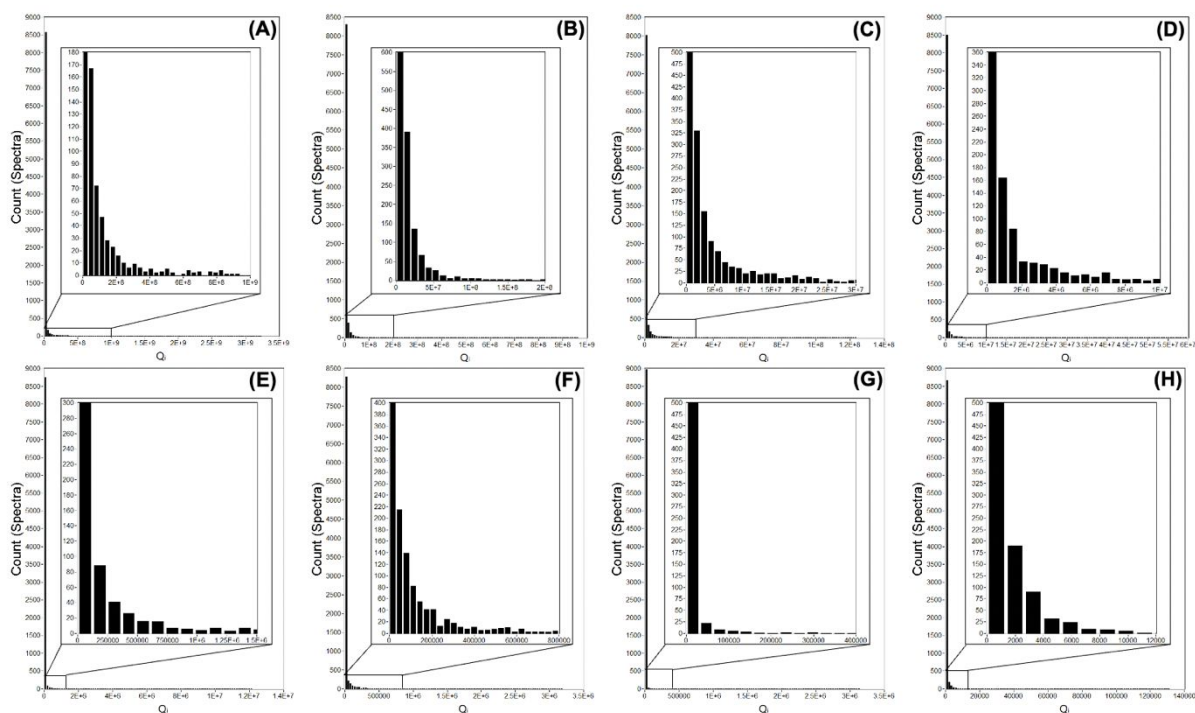

Fig. S3. Histograms showing the Q_i distribution of all acquired spectra for each TFV concentration in the single deposition experiment. (A) 500 ng/mL; (B) 400 ng/mL; (C) 200 ng/mL; (D) 100 ng/mL; (E) 50 ng/mL; (F) 40 ng/mL; (G) 25 ng/mL; (H) Blank (Milli-Q H_2O). Zoomed insets of clustered data for each concentration are shown for clarity. Histograms were generated using 100 bins where the count of spectra in each bin was plotted as a function of Q_i whose width corresponds to the Q_i range of spectra in the bin.
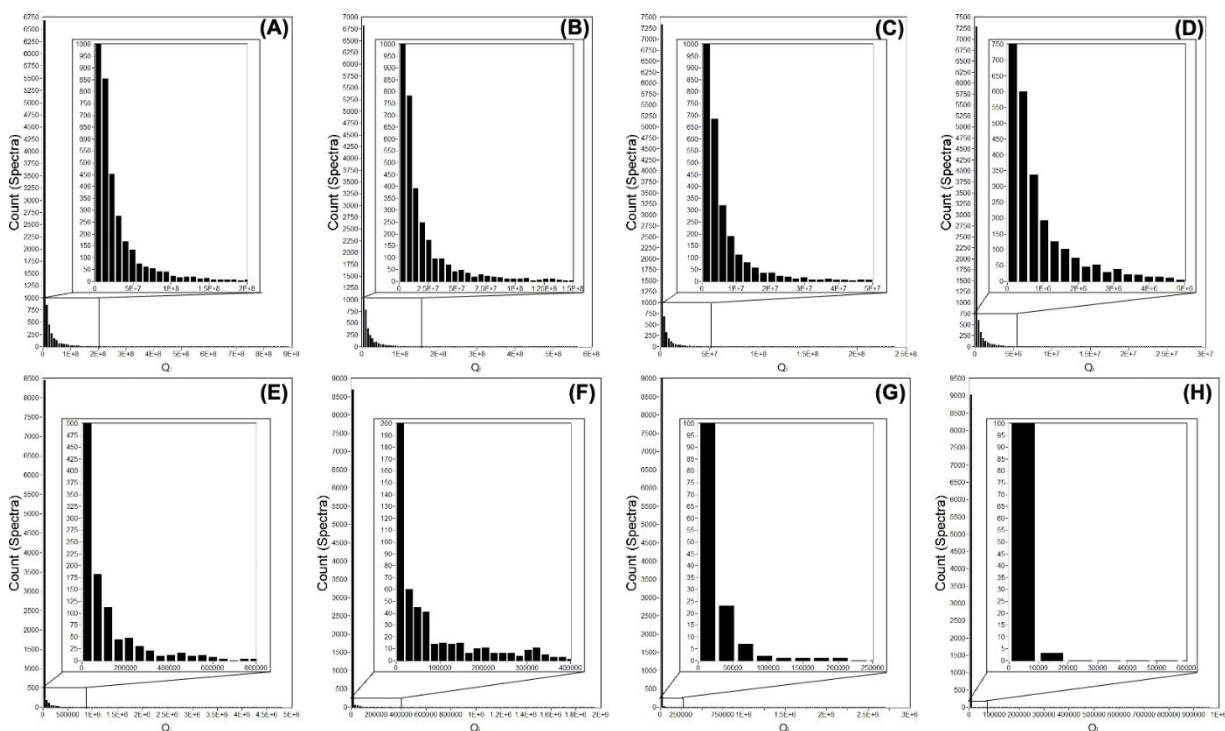
Fig. S4. Histograms showing the Q_i distribution of all acquired spectra for each TFV concentration in the double deposition experiment. (A) 500 ng/mL; (B) 400 ng/mL; (C) 200 ng/mL; (D) 100 ng/mL; (E) 50 ng/mL; (F) 40 ng/mL; (G) 25 ng/mL; (H) Blank (Milli-Q H_2O). Zoomed insets of clustered data for each concentration are shown for clarity. Histograms were generated using 100 bins where the count of spectra in each bin was plotted as a function of Q_i whose width corresponds to the Q_i range of spectra in the bin.
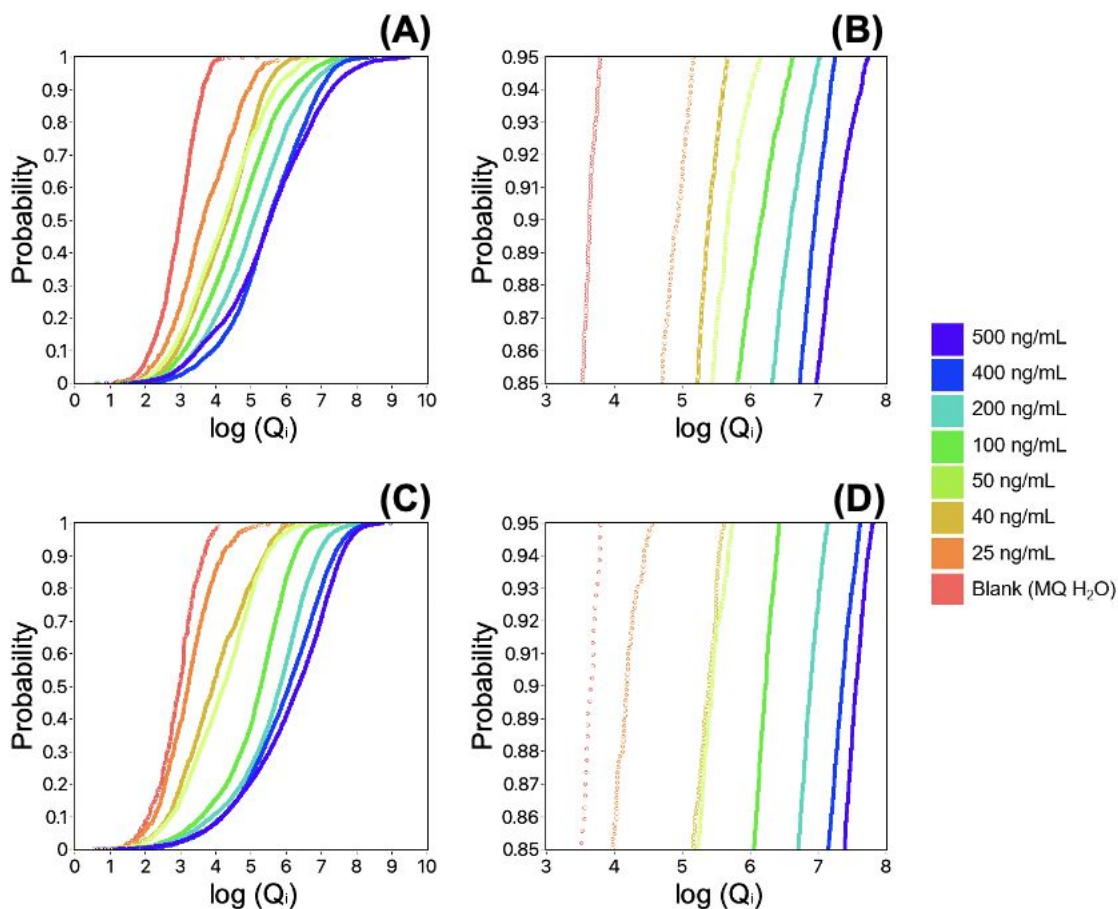
Fig. S5. Unfitted CDFs of the (A) single deposition experiment and (B) probability range 0.85 – 0.95 zoomed in for clarity. (C) and (D) show this same analysis for the double deposition experiment.

Table S1. Standard deviation (σ) of Σ ΔCDF values for each TFV concentration from the single and double deposition datasets. The difference between the σ of the single deposition and double deposition was calculated for each concentration, and averaged.

[TFV] (ng/mL)	σ single deposition	σ double deposition	σ difference (single - double)
500	0.1095	0.0646	0.0449
400	0.0759	0.0753	0.0006
200	0.1035	0.0789	0.0246
100	0.1363	0.0312	0.1051
50	0.0585	0.0783	-0.0198
40	0.1224	0.0992	0.0233
25	0.0908	0.1250	-0.0342
		Average σ difference	**0.0206**